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(54) Title: PROCESS FOR EXTRACTING ESTROGENS FROM PREGNANT MARE URINE (PMU)

(57) Abstract: The present invention relates to a process for extracting estrogens from pregnant mare urine (PMU) comprising the following steps: (a) adding a salt to the pregnant mare urine, (b) conducting a liquid-liquid extraction of the pregnant mare urine of step (a) by using an organic solvent which is immiscible with the pregnant mare urine, and (c) separating the organic extract and drying it to form an extract. The operation of the present invention is easy and efficient. The concentrating efficiency is high, which enables convenient transportation of pregnant mare urine and is economical. In addition, the amount of conjugated estrogens recovered from the extract of pregnant mare urine is high.

PROCESS FOR EXTRACTING ESTROGENS FROM PREGNANT MARE URINE (PMU)

FIELD OF THE INVENTION

The present invention relates to a process for extracting estrogens from pregnant mare urine (PMU).

BACKGROUND OF THE INVENTION

Conjugated estrogens are effective drugs for treating the symptoms of menopause. The natural source of conjugated estrogens may come from the extraction of pregnant mare urine (PMU).

U.S. Patent No. 2,834,712, issued to American Home Products Company in 1958, discloses a process for preparing substantially pure estrogenic mixtures with low toxicity. Said process primarily utilizes the principle that substances will exhibit different solubilities in different solutions under different pH values to carry out the liquid-liquid extraction. The purification of pregnant mare urine thereby separates estrogenic mixtures from pregnant mare urine. The procedure disclosed in this patent is quite complicated and the solvents added in the process (such as benzene and hexane) are not suitable for pharmaceutical formulations. In addition, the amount of estrogenic mixtures recovered from pregnant mare urine is limited.

U.S. Patent Nos. 5,723,454 and 5,814,624, issued to Solvay Deutschland GmbH in 1998, discloses a method for obtaining a natural mixture of conjugated estrogens from pregnant mare urine. The method primarily utilizes a solid-liquid extraction to separate the desired conjugated estrogens from pregnant mare urine. The disadvantages of this method lie in the use of a column separation. The Amberlite resin packed in the column has limited life and high cost. Also, the process is timeconsuming. In addition, the amount of pregnant mare urine which can be treated is quite limited.

To overcome the problems encountered in the art as delineated in the above, the present invention provides a process for extracting estrogens from pregnant mare urine (PMU). The operation of the present invention is simple and efficient. Moreover, the extraction efficiency is high, which enables convenient transportation of pregnant mare urine, and is economical. Finally, the amount of conjugated estrogens recovered from the extract of pregnant mare urine is high.

SUMMARY OF THE INVENTION

The present invention provides a process for extracting estrogens from pregnant mare urine (PMU). It involves the addition of a salt and a liquid-liquid extraction using an organic solvent which is immiscible with pregnant mare urine.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a process for extracting estrogens from pregnant mare urine (PMU) comprising the following steps:

(a) adding a salt to the pregnant mare urine,

(b) conducting a liquid-liquid extraction of the pregnant mare urine of step (a) by using an organic solvent which is immiscible with the pregnant mare urine, and

(c) separating the organic extract and drying it to form an extract.

The main principles of the present invention essentially lie in the addition of a salt to lower the solubility of conjugated estrogens in an aqueous phase, and the liquid-liquid extraction to partition the majority of conjugated estrogens into the organic phase. Therefore, the conjugated estrogens are separated with proteins and phenolic materials abundant in the aqueous phase and a primary purification is accomplished. The ionic strength of the aqueous phase is changed due to the presence of salt. This leads to the formation of chemical bonds between conjugated estrogens and cations. A small amount of covalent materials precipitate in the aqueous solution, while the majority of conjugated estrogens selectively enter the organic phase which is immiscible with pregnant mare urine and is separated from proteins and phenolic compounds. After the organic phase is separated from the aqueous phase, the organic solvent is evaporated. An extract of pregnant mare urine with an extremely high concentration of conjugated estrogens is thus obtained.

In the process of the present invention, the pregnant mare urine may be pre-treated in a known manner to remove solid impurities, such as by a filtration or a centrifugation.

The salt suitable for use in step (a) can be any organic or inorganic salt which is known to persons skilled in the art. When the salt is an organic salt, it can be selected from the group consisting of tetra ammonium salts and mixtures thereof; when the salt is an inorganic salt, it can be, for example, one expressed in the formula M_xA_y, wherein M is selected from the group consisting of alkali metals and earth alkali metals; A is selected from the group consisting of halogen, hydroxyl, a carbonate ion, a sulfate ion, a sulfite ion, a phosphate ion and a nitrate ion; each of x and y represents a positive integer of 1, 2 or 3. Preferably, the salt is selected from the group consisting of sodium chloride, potassium chloride, magnesium chloride, sodium hydroxide, potassium hydroxide, magnesium hydroxide, sodium sulfate, potassium sulfate, magnesium sulfate, tetramethyl ammonium chloride, tetraethyl ammonium chloride and mixtures thereof.

Step (b) is a liquid-liquid extraction step carried out by using an organic solvent which is immiscible with the pregnant mare urine. The organic solvent suitable for use in step (b) can be any one known to person skilled in the art, such as that selected from the group consisting of C_4-C_6 alkanes, C_1-C_6 haloalkanes, C_2-C_8 ethers, C_3-C_8 ketones, C_2-C_6 esters, C_4-C_8 alcohols, cyclic C_4-C_8 alcohols, cyclic C_3-C_8 ketones, C_1-C_8 amides, C_6-C_{12} aryl hydrocarbons, furans, pyridines, nitriles and mixtures thereof. Preferably, the organic solvent is selected from the group consisting of ethyl acetate, acetone, tetrahydrofuran (THF), acetonitrile, cyclohexanol and mixtures thereof.

The operating conditions for carrying out the process of the present invention can be determined and adjusted by persons skilled in the art based on their own knowledge in this field under the guidelines of published patents and the disclosure of the present invention. For example, the amount of the salt in step (a) can be that which allows its concentration in pregnant mare urine to reach the range of 0.5 M to a saturated concentration, preferably 1 M to a saturated concentration. Step (a) can be conducted at a temperature range of 5℃ to the temperature at which the solubility of the salt is optimized. Preferably, step (a) is carried out at a temperature of 5℃ to 55 ℃.

Step (b) can be carried out under neutral to basic conditions. Preferably, the pH value of the solution is adjusted to a range between 7 and 9. Moreover, the ratio of the organic solvent and the pregnant mare urine of step (b) can be within the range of 1:1 to 8:1, preferably 1:1 to 6:1. Optionally, a surfactant conventionally known to persons skilled in the art can be incorporated into step (b) to eliminate the foaming condition and the emulsion layer which occurs during the liquid-liquid extraction. Surfactants suitable for use in the process of the present invention can be cationic, anionic or amophoteric surfactants. Preferred surfactants are quaternary ammonium salts or sulfonic salts.

The extract of pregnant mare urine obtained from the process of the present invention can be subjected to a series of purification and separations by conventional methods to separate the conjugated estrogens contained therein. Surprisingly, the inventor found that the recovery rate of the conjugated estrogens from the concentrate of pregnant mare urine in accordance with the process of the present invention can be from 30% to 90%.

An advantage of the present invention is that its operation is easy and efficient. The extraction efficacy is good, which enables convenient transportation of pregnant mare urine and is economical. In addition, the amount of conjugated estrogens recovered from the extract of pregnant mare urine is high.

The following embodiments are provided to demonstrate the feasibility of the present invention, to substantiate the technical contents of the invention, and to allow persons skilled in the art to understand the contents of the invention and to practice it. They are not intended to limit the reasonable scope of protection. Various improvements and obvious modifications that can be accomplished easily by persons skilled in the art based on the teachings of conventional techniques are all within the scope of the present invention.

EXAMPLE

Example 1

2 M sodium chloride solution is prepared by adding 116 g of sodium chloride into 1 liter of pregnant mare urine at 25℃. The solution is well stirred. When the precipitation is balanced, 2 liter of ethyl acetate is added into the solution. The ratio of organic phase to aqueous phase is 2:1. A liquid-liquid extraction is conducted. The organic phase is separated and the organic solvent is removed. Then, the residue is dried. The dried

material is dissolved in a mixed solution of acetonitrile and water. HPLC analysis reveals that the dried material contains 73% of conjugated estrogens originally contained in the pregnant mare urine.

Examples 2 to 19

The procedure of Example is repeated under the conditions shown in Table 1 recited below. The recovery rate is also given in Table 1.

Table 1

Example No.	Salts	Organic solvent	H_2O/Org. solvent	Temperature (°C)	Recovery Rate (%)
2	4M NaCl	ethyl acetate	1/2	room temp.	83
3	2M NaCl	ethyl acetate	1/4	room temp.	87
4	0.5M NaCl	ethyl acetate	1/2	room temp.	34
5	2M NaCl	ethyl acetate	1/1	room temp.	61
6	2M KCl	ethyl acetate	1/2	room temp.	78
7	2M NaOH	ethyl acetate	1/2	room temp.	56
8	2M KOH	ethyl acetate	1/2	room temp.	85
9	2M $MgSO_4$	ethyl acetate	1/2	room temp.	71
10	2M $CaCl_2$	ethyl acetate	1/2	room temp.	47
11	2M $(CH_3)_4NCl$	ethyl acetate	1/2	room temp.	25
12	2M NaCl	CH_3CN	1/2	room temp.	89
13	2M NaCl	THF	1/2	room temp.	90
14	2M NaCl	acetone	1/2	room temp.	91
15		ethyl acetate	1/2	room temp.	18
16	2M NaCl	ethyl acetate	1/1	room temp.	58
17	2M NaCl	ethyl acetate	1/2	5	68
18	2M NaCl	ethyl acetate	1/2	35	73
19	2M NaCl	ethyl acetate	1/2	55	67

WHAT IS CLAIMED IS:

1. A process for extracting estrogens from pregnant mare urine (PMU) comprising the following steps:

(a) adding a salt to the pregnant mare urine,

(b) conducting a liquid-liquid extraction of the pregnant mare urine of step (a) by an organic solvent which is immiscible with the pregnant mare urine, and

(c) separating the organic extract and drying it to form an extract.

2. A process according to claim 1, wherein the salt of step (a) is an inorganic salt of the formula M_xA_y, in which M is selected from the group consisting of alkali metals and earth alkali metals, A is selected from the group consisting of halogen, hydroxyl, a carbonate ion, a sulfate ion, a sulfite ion, a phosphate ion and a nitrate ion, and x and y each represents a positive integer which is 1, 2 or 3.

3. A process according to claim 1, wherein the salt of step (a) is an organic salt, it can be selected from the group consisting of tetra ammonium salts and mixtures thereof.

4. A process according to claim 2 or 3, wherein the salt of step (a) is selected from the group consisting of sodium chloride, potassium chloride, magnesium chloride, sodium hydroxide, potassium hydroxide, magnesium hydroxide, sodium sulfate, potassium sulfate, magnesium sulfate, tetramethyl ammonium chloride, tetraethyl ammonium chloride and mixtures thereof.

5. A process according to claim 1, wherein the amount of the salt in step (a) is that which allows its concentration in pregnant mare urine to reach the range of 0.5 M to a saturated concentration.

6. A process according to claim 4, wherein the amount of the salt in step (a) is that which allows its concentration in pregnant mare urine to reach the range of 1 M to a saturated concentration.

7. A process according to claim 1, wherein the organic solvent which is immiscible with the pregnant mare urine of step (b) is selected from the group consisting of C_4-C_6 alkanes, C_1-C_6 haloalkanes, C_2-C_8 ethers, C_3-C_8 ketones, C_2-C_6 esters, C_4-C_8 alcohols, cyclic C_4-C_8 alcohols, cyclic C_3-C_8 ketones, C_1-C_8 amides, C_6-C_{12} aryl hydrocarbons, furans, pyridines, nitriles and mixtures thereof.

8. A process according to claim 7, wherein the organic solvent which is immiscible with the pregnant mare urine of step (b) is selected from the group consisting of ethyl acetate, acetone, tetrahydrofuran (THF), acetonitrile, cyclohexanol and mixtures thereof.

9. A process according to claim 1, wherein step (b) is carried out under a neutral to basic condition.

10. A process according to claim 1, wherein the ratio of the organic solvent and the pregnant mare urine of step (b) is in the range of 1:1 to 8:1.

11. A process according to claim 1, which further comprises an additional step, so as to further purify conjugated estrogens from the extract.

INTERNATIONAL SEARCH REPORT

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PCT/US99/23730

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07J 9/00; A61K 35/22
US CL :552/545; 424/546
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 552/545; 424/546

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS, USPATFUL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2,711,988 A (DEANS et al) 28 June 1955, see the entire article, especially claims 1-3.	1-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

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